Remarks/Arguments

Applicant respectfully requests favorable reconsideration of the subject application, particularly in view of the above amendment and the following remarks. There is no additional fee for the above amendment because the number of independent claims remains unchanged and the total number of claims has been reduced.

Applicant has amended the specification to more correctly identify the drawings previously identified as Fig. 2 and Fig. 3 as Figs. 2A-2D and Figs. 3A-3D.

Applicant has amended Claim 1 to indicate that only one bacterial cell is isolated from the environmental sample comprising a plurality of microorganisms. Claim 1 has also been amended to incorporate the limitations of Claim10, thereby including the further limitation that the isolation of the bacterial cell is carried out using flow cytometry. Claim 1 has still further been amended to clarify that it is a DNA fragment that has been amplified and cloned which is then sequenced.

Claim 2 has been amended to correct an obvious typographical error. In particular, the term "polymeric" has been deleted and the term "polymerase" has been inserted in its place. This correction is considered by Applicant to be obvious since the term "PCR", also recited in the claim, is known to refer to "polymerase chain reactions."

Claim 5 has been amended to clarify that the at least one universal primer is either a high-GC content primer or a high-AT content primer. By virtue of the incorporation of the limitations of Claim 10 into Claim 1, Claims 9 and 10 have been canceled from the subject application. Claim 14 has been amended to correct an obvious grammatical error. Claim 17 has been amended to indicate the existence of an antecedent basis in Claim 1 for the element "flow cytometry." And, finally, Claim 18 has been canceled as being directed to a non-elected species.

Claims 1-17 and 19-21 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiner has indicated that Claim 1 as originally filed is not commensurate in scope with the specification in that it is not limited to requiring that the sequencing step utilizes material, such as DNA, that was first either cloned or amplified. In response thereto, Applicant has amended Claim 1 to clarify that it is an amplified and cloned DNA fragment from a single bacterial cell which is sequenced.

Claim 5 has been indicated to be vague and indefinite because the "at least one universal primer" is required to have both a high-GC and high-AT content which conflict in that nucleic acid sequences are one or the other but not both. In response thereto, Applicant has amended Claim 5 to clarify that the at least one

universal primer is either a high-GC content primer or a high-AT content primer. Applicant respectfully urges that the above amendment overcomes the rejection under 35 U.S.C. 112, second paragraph.

The invention claimed by Applicant is a method for identifying unculturable microorganisms in which *one* bacterial cell is isolated from an environmental sample comprising a plurality of microorganisms using flow cytometry. At least one DNA fragment from the one bacterial cell is amplified, forming at least one amplified DNA fragment, which at least one amplified DNA fragment is cloned into at least one *E. coli* vector. The at least one amplified and cloned DNA fragment is then sequenced, resulting in identification of at least one DNA sequence, which is then compared with existing DNA databases, resulting in identification of said at least one DNA sequence as either an unculturable microorganism or a known microorganism. *The crux of this invention is the isolation of a single bacterial cell using flow cytometry.* Applicant respectfully urges that, for the reasons set forth herein below, the prior art relied upon by the Examiner for rejection of the subject application neither teaches nor suggests such a method as claimed by Applicant.

Claims 1 and 8 have been rejected under 35 U.S.C. 102(e)(2) as being anticipated by Short, U.S. Patent 5,958,672 (hereinafter "the Short patent"). This

rejection is respectfully traversed. The Short patent teaches a process of screening clones having DNA from an uncultivated microorganism for a specified protein, which process comprises screening for a specified protein activity in a library of clones prepared by (i) recovering DNA from a DNA population derived from at least one uncultivated microorganism; and (ii) transforming a host with recovered DNA to produce a library of clones which are screened for the specified protein activity. The library is produced from DNA which is recovered without culturing of an organism, particularly where the DNA is recovered from an environmental sample containing microorganisms which are not or cannot be cultured (Col. 1, lines 28-42). Although the Short patent does teach recovering DNA from a DNA population derived from at least one uncultivated microorganism, *it neither teaches nor suggests isolation of a single bacterial cell using flow cytometry as claimed by Applicant*. Indeed, the Short patent indicates the source of such uncultivated microorganism is *a mixed population of uncultured organisms* (See Claim 6), in direct contrast to the invention claimed by Applicant which utilizes a single bacterial cell from an environmental sample. Accordingly, given that the Short patent neither teaches nor suggests isolation of a single bacterial cell using flow cytometry, Applicant respectfully urges that the Short patent does not anticipate the invention claimed by Applicant in the manner required by 35 U.S.C. 102(e)(2).

Claims 1, 2, 5-8 and 16 have been rejected under 35 U.S.C. 103(a) as being unpatentable over the Short patent in view of Goh et al., U.S. Patent 5,708,160. This rejection is respectfully traversed. Applicant's arguments with respect to the Short patent are equally applicable to this rejection and, thus, will not be repeated. The Goh et al. patent teaches a method of using oligonucleotide primers for identifying the species of an organism, wherein the identification includes amplification of a variable polynucleotide sequence encoding a highly conserved region of a heat shock polypeptide. The Goh et al. patent is relied upon by the Examiner as teaching the use of a highly conserved region of a polypeptide as a universal primer. Thus, the Examiner argues that it would have been obvious to one of ordinary skill in the art at the time of Applicant's invention to identify microorganisms via protein sequence as in the Short patent including motivating PCR practice wherein protein sequences are identified for microorganism identification by the use of universal primers, thereby resulting in the universal primer utilizing embodiments of the invention claimed by Applicant. Applicant respectfully urges, however, that neither the Short patent nor the Goh et al. patent teaches or suggests isolation of a single bacterial cell using flow cytometry as claimed by Applicant. Given the absence of these elements, Applicant respectfully urges that the combination of the teachings of the Short patent and the Goh et al. patent would not

lead one skilled in the art to the method of the invention claimed by Applicant. Accordingly, Applicant respectfully urges that the Short patent and the Goh et al. patent, alone or in combination, do not render Applicant's claimed invention obvious in the manner required by 35 U.S.C. 103(a).

Claims 1, 3-8 and 15 have been rejected under 35 U.S.C. 103(a) as being unpatentable over the Short patent in view of Hartley, U.S. Patent 5,043,272 (hereinafter "the Hartley patent"). This rejection is respectfully traversed. Applicant's arguments with respect to the Short patent are equally applicable to this rejection and, thus, will not be repeated other than to reiterate that *the Short patent neither teaches nor suggests a method for identifying unculturable microorganisms in which a single bacterial cell is isolated from an environmental sample comprising a plurality of microorganisms using flow cytometry as claimed by Applicant.* The Hartley patent teaches a process for substantially amplifying template nucleic acid present in a sample wherein the amplification may be performed without prior knowledge of specific sequences, which process comprises apposition of random oligonucleotide primers to said template nucleic acid under conditions such that extension products of the primers are synthesized which are complementary to the template nucleic acid. The Hartley patent is relied upon by the Examiner as teaching generic amplification of any desired DNA sample via random primer usage and as

teaching that such random primer practice is universally useful in amplifying any DNA for later analysis as required by the Short patent. The Examiner further states that this random and, thus, universal primer practice is motivated for making the amplification of DNA via PCR generically useful without being limited as to source. The Examiner further indicates that such random priming will produce fragments from multiple loci as recited in Claim 15 of the subject application. Thus, the Examiner argues that it would have been obvious to one of ordinary skill in the art to utilize the improvement of the Hartley patent directed to random and arbitrary universal PCR priming in any desired method where amplification of DNA is desired as taught by the Short patent to result in the arbitrary primed amplification embodiments of the above listed claims. Applicant respectfully urges, however, that *neither the Short patent nor the Hartley patent teaches or suggests isolation of a single bacterial cell using flow cytometry as claimed by Applicant.* Thus, Applicant further respectfully urges that the Short patent and the Hartley patent, alone or in combination, do not render Applicant's claimed invention obvious in the manner required by 35 U.S.C. 103(a).

Regarding the Information Disclosure Statement filed by Applicant, the Examiner indicates that two of the references cited therein had unrecognizable citation numbers and, thus, could not be considered. In response thereto, Applicant is filing

another Information Disclosure Statement herewith and resubmitting the references in question for consideration by the Examiner.

The Examiner has noted certain informalities in the subject application and has required appropriate correction thereof. In particular, the Examiner indicates that the BRIEF DESCRIPTION OF THE DRAWINGS section in the specification on Page 8 does not summarize each drawing separately. In particular, the Examiner indicates that there is no Figure 2 or 3 in the drawings, but rather Figures 2A-2D and 3A-3D. In response thereto, Applicant has amended the specification to reference Figs. 2A-2D and 3A-3D.

The Examiner has also noted that the word "polymeric" appears in Claim 2, line 2 to be misspelled because PCR is commonly known as "polymerase" chain reaction. In response thereto, Applicant has amended Claim 2 as described herein above to address this informality.

Conclusion

Applicant intends to be fully responsive to the outstanding Office Action. If the Examiner detects any issue which the Examiner believes Applicant has not addressed in this response, Applicant urges the Examiner to contact the undersigned.

Applicant sincerely believes that this patent application is now in condition for allowance and, thus, respectfully requests early allowance.

Respectfully submitted,

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